## Coupled enzymatic reaction system using a formate dehydrogenase derived from *Candida boidinii*

The present invention relates to a coupled reaction system
5   operating enzymatically which is distinguished in that it
operates in a solvent mixture having two phases. In
particular, the invention is directed towards a reaction
system comprising a cofactor-dependent enzymatic
transformation of an organic compound and an enzymatic
10   cofactor regeneration using a formate dehydrogenase derived
from *Candida boidinii* (or mutants based thereon) in the
same system.

The isolation of optically active organic compounds, for
example alcohols and amino acids, by biocatalytic means is
15   gaining increasingly in importance. The coupled use of two
dehydrogenases with cofactor regeneration has proved to be
a method for the large-scale industrial synthesis of these
compounds (DE 197 53 350).

Scheme 1:
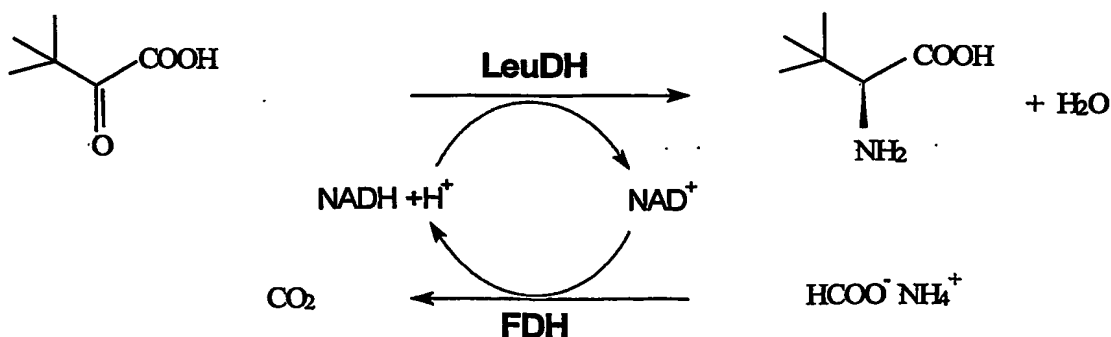
In situ regeneration of NADH with the NAD-dependent formate
dehydrogenase derived from *Candida boidinii* in the course
of the reductive amination of trimethyl pyruvate to L-

pseudoleucine (Bommarius et al. Tetrahedron Asymmetry 1995,
6, 2851-2888).

Besides their catalytic property and efficiency, the
biocatalysts that are employed efficiently in the aqueous
5   medium additionally have the advantage, in contrast with a
large number of synthetic metalliferous catalysts, that the
use of metalliferous feed materials, in particular feed
materials that contain heavy metal and are consequently
toxic, can be dispensed with. The use of expensive and, in
10 addition, hazardous reducing agents such as borane, for
example, in the course of the asymmetric reduction can also
be dispensed with.

However, difficulties arise in the course of the conversion
of substrates that are sparingly soluble in water. Similar
15 difficulties exist in connection with sparingly water-
soluble products. This is the case, in particular, in the
preparation of optically active alcohols in accordance with
the above concept, since the ketones that are required as
starting compounds have a clearly lower solubility than the
20 α-keto acids employed in Scheme 1.

One conceivable solution in principle would be the
implementation of the biocatalytic reduction using an
alcohol dehydrogenase and a formate dehydrogenase in a
polar organic solvent or in an aqueous solution thereof.
25 In this case, both the enzymes and the substrate and
optionally the product should be soluble. A general
disadvantage of a direct presence of an organic solvent,
however, is constituted by the considerable diminution of
the enzyme activity which generally occurs under these
30 conditions (see, for example, Anderson et al., *Biotechnol.
Bioeng.* 1998, *57*, 79-86). In particular, the formate
dehydrogenase derived from *Candida boidinii*, being the only
NADH regeneration enzyme employed hitherto on an industrial
scale and available in commercial quantities, regrettably
35 exhibits high sensitivity to organic solvents

(EP 1 211 316). This is also shown in Comparative
Example 1 with the use of DMSO, sulfolane, MTBE, acetone,
isopropanol and ethanol etc. by way of organic solvent
component in supplemental amounts of, in each case, only
5   10 % by volume (see Fig. 1).

Various approaches are known with a view to solving this
problem relating to a stabilisation of the formate
dehydrogenase derived from *Candida boidinii* in the presence
of organic solvents, for example the implementation of
10 reactions through additional use of surfactants by way of
surface-active substances. But the rate of reaction that
is diminished by a factor of approximately 40 (!) and also
the inhibition of the formate dehydrogenase which occurs
(B. Orlich et al., *Biotechnol. Bioeng.* 1999, *65*, 357-362.)
15 prove to be disadvantageous in this case. The authors note
in addition that, by reason of the low stability of the
alcohol dehydrogenase, a reduction process under these
conditions of a microemulsion is not economic. The same
also holds, in principle, for the method described in
20 EP 340 744, in which lyotropic mesophases were chosen as
reaction site in the presence of an aqueous and/or organic
phase.

A further basic possibility for the implementation of
biocatalytic reactions consists in the application of
25 immobilised enzymes in the organic solvent or the use of
enzymes in a homogeneous solution consisting of water and a
water-miscible organic solvent. However, the successes
with these techniques, in which a direct contact of organic
solvent and enzyme occurs, are limited to a few enzyme
30 classes, in particular hydrolases. For instance, in
DE 44 36 149 it is noted that the "direct presence of
organic solvents (water-miscible or non-water-miscible) is
tolerated only by a few enzymes that pertain to the class
of the hydrolases." Although a few further examples from
35 other enzyme classes have become known in the meantime
(*inter alia*, oxynitrilases and an FDH derived from yeast),

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the statement made in DE 44 36 149 continues to have
validity for the majority of enzymes. For instance, an
efficient immobilisation of the FDH derived from *Candida
boidinii* is not known. In addition, the immobilisation

5   itself is associated with additional costs due to the
immobilisation step as well as the immobilisation
materials.

Therefore processes have been developed industrially that
avoid the presence of organic solvents by reason of the

10 risk of the deactivation or denaturation of the enzymes.
For instance, DE 44 36 149 describes a process in which the
product is extracted from the reaction solution into an
organic solvent through a product-permeable membrane, in
particular a hydrophobic membrane. Compared with a

15 standard process in a stirred-tank reactor, however, this
process is technically clearly more elaborate; besides, the
requisite organic membranes are also an additional cost
factor. Furthermore, this method is only suitable for
continuous processes. Moreover, it is a disadvantage that

20 the achievable space-time yields with this procedure are
comparatively low. For example, in the course of the
reduction of acetophenone a space-time yield of only
88 g/(L*d) is obtained (S. Rissom et al., *Tetrahedron:
Asymmetry* 1999, *10*, 923-928). In this regard it is to be

25 noted that acetophenone itself is a relatively well water-
soluble ketone, and most analogue substituted acetophenone
ketones and related ketones possess far lower solubilities,
so that the space-time yield for typical hydrophobic
ketones should be distinctly lower. Despite these

30 considerable disadvantages, this process is regarded as the
hitherto preferred method for the asymmetric biocatalytic
reduction of sparingly soluble ketones using isolated
enzymes (see also: A. Liese, K. Seelbach, C. Wandrey,
Industrial Biotransformations, Wiley-VCH Verlag, Weinheim,

35 2000, pp. 103-106).

To sum up, it may consequently be noted that at the moment no process is known that helps to circumvent the disadvantages listed above and permits the enzymatic preparation of sparingly water-soluble substrates on an
5   industrial scale using a formate dehydrogenase derived from *Candida boidinii* (or mutants based thereon) in the "direct" presence of organic solvents.

The object of the present invention was therefore to specify a possibility as to how, in particular, sparingly
10   water-soluble organic compounds can be made available to a coupled cofactor-dependent enzymatic conversion to such a sufficient extent that an application of the conversion on an industrial scale can be undertaken under economically and ecologically advantageous conditions. In particular,
15   one object was that such a process should be suitable for the reduction of sparingly water-soluble ketones and should permit the use of the formate dehydrogenase derived from *Candida boidinii* in the "direct" presence of organic solvents (i.e. without separation by a hydrophobic
20   membrane).

This object is achieved in the manner defined in the Claims. Claims 1 to 8 are directed towards a reaction system operating in accordance with the invention. Claim 9 protects a device. Claim 10 relates to a process operating
25   in accordance with the invention, whereas Claims 11 and 12 are directed towards preferred uses of the reaction system according to the invention.

By virtue of the fact that a coupled enzymatic reaction system is made available comprising an NADH-dependent
30   enzymatic transformation of an organic compound with an alcohol dehydrogenase and an enzymatic regeneration of the NADH with the formate dehydrogenase derived from *Candida boidinii* or mutants thereof in a two-phase solvent system in which an aqueous phase is in contact with a liquid
35   organic phase, the solution to the stated object is

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attained, in particular in a manner that is surprising, by
no means foreseeable and, according to the invention,
particularly advantageous. Contrary to the opinion that
can be deduced from the state of the art, it is
5 surprisingly possible, despite the presence of an organic
solvent, to allow the coupled enzymatic reaction system to
operate without loss of activity, due to the solvent, of
one of the enzymes, in particular of the formate
dehydrogenase derived from Candida boidinii, with space-
10 time yields that are sufficient for the industrial scale.
The FDH originates from the organism Candida boidinii
itself, or further developed rec-mutants of the same
organisms may also be employed (DE 197 53 350). Quite
particularly advantageous is the use of the mutant that
15 exhibits the C23S/C262A amino-acid substitutions.
Particularly surprising in this connection is the fact that
the formate dehydrogenase derived from C. boidinii, despite
the high instability in relation to organic solvents that
has been observed (see Comparative Example 1 in the
20 Experimental Part), can be employed very efficiently under
these conditions.

The organic solvent that is employed in the reaction system
is intended to form - as explained above - two separate
phases with the aqueous phase that is present. Within the
25 bounds of this requirement, a person skilled in the art is,
in principle, free in the choice of the organic solvent.
However, it has proved to be advantageous if by way of
organic phase a solvent is chosen that possesses a
solubility in water that is as low as possible (logP value
30 ≥ 3, preferably ≥ 3.1, more preferably ≥ 3.2 etc.). Since
the organic solvent is also intended to take up the
sparingly water-soluble educt at the same time, it is also
important furthermore that said solvent possesses a
solubility in respect of the organic compounds employed
35 that is as high as possible.

Organic solvents of such a type, which can be
preferentially employed in the reaction system, are
aromatic or aliphatic hydrocarbons that are liquid under
the given reaction conditions. In particular, n-pentane,
5   n-hexane, n-heptane, n-octane, isooctane, cyclohexane,
methylcyclohexane and also branched-chain isomers thereof
are quite particularly preferred. Halogenated hydrocarbons
can also be employed ($CHCl_3$, CH_2Cl_2, chlorobenzene etc.).
By way of aromatic hydrocarbons, toluene, xylenes or
10 benzene enter into consideration.

The quantitative ratio of organic solvent to aqueous
portion can be chosen arbitrarily. The organic solvent is
employed in a quantity relative to the total volume
amounting to 5-80 vol.%, preferably 10-60 vol.%,
15 particularly preferably around 50 vol.%.

Contrary to the approach that is proposed in the state of
the art, namely of adding surfactants to the enzymatic
reaction mixture in order to accelerate the enzymatic
transformation, in which phase transitions in the course of
20 the reaction are minimised, the present invention provides
evidence that the use of a reaction system according to the
invention proceeds particularly successfully when the
system contains no surfactants.
The term 'surfactants' in this context is understood to
25 mean all those substances which are capable of building up
micellar structures or of lowering the surface tension at
liquid-liquid phase boundaries.

As already indicated, the concentration with which the
substrates are employed in the reaction system should be
30 such that a conversion can be effected that is advantageous
from economic viewpoints. The organic compound should
therefore be present prior to the start of the reaction
advantageously in a concentration of > 25 mM, preferably
> 100 mM, particularly preferably > 200 mM and quite
35 particularly preferably > 500 mM per L of total volume of

the solvents (= sum of the volumes of organic solvent and
aqueous portion). An upper limit for the concentration is
constituted naturally by the guarantee of the viability of
the reaction; in particular, stirrability of the reaction
5   mixture should obtain in every case. However, working may
preferably take place also above the saturation limit for
the substrate or the product.

The concrete coupled enzymatic reaction system can,
according to the invention, be employed in all enzymatic
10 reactions coming into consideration by a person skilled in
the art for this purpose in which keto groups are
transformed into alcohol groups. Preferred, however, are
oxidoreductase reactions, as stated. The process is
suitable for the use of any types of alcohol dehydrogenase.
15 The alcohol dehydrogenases that are employed in accordance
with the invention preferably originate from the organisms
Rhodococcus erythropolis (S-ADH) or Lactobacillus kefir (R-
ADH) *(ADH derived from R. erythropolis: J. Peters, T.
Zelinski, M.-R. Kula, Purification and characterization of*
20 *a novel carbonyl reductase silated from Rhodococcus*
erythropolis, J. Biotechnol. 1994, 33, 283-292) (ADH derived
from Lactabacillus kefir: C. W. Bradshaw, W. Hummel, C.-H.
Wong, Lactobacillus kefir Alcohol Dehydrogenase: A Useful
Catalyst for Synthesis, J. Org. Chem. 1992, 57, 1532-
25 *1536.).*

In a next development the present invention relates to a
device for the transformation of organic compounds that
comprises the reaction system according to the invention.
Devices to be employed advantageously are, for example, the
30 stirred tank or stirred-tank cascades, or membrane reactors
that can be operated both in batch operation and
continuously.

Within the scope of the invention the term 'membrane
reactor' is understood to mean any reaction vessel in which
35 the catalyst is enclosed in a reactor while low-molecular

substances are supplied to the reactor or are able to leave
it. In this connection the membrane may be integrated
directly into the reaction chamber or may be installed
outside in a separate filtration module wherein the
5   reaction solution flows continuously or intermittently
through the filtration module and the retentate is recycled
into the reactor. Suitable embodiments are described,
inter alia, in WO 98/22415 and in Wandrey et al. in
Jahrbuch 1998, Verfahrenstechnik and Chemieingenieurwesen,
10   VDI p 151 ff.; Wandrey et al. in Applied Homogeneous
Catalysis with Organometallic Compounds, Vol. 2, VCH 1996,
p 832 ff.; Kragl et al., Angew. Chem. 1996, 6, 684 f.
The continuous mode of operation which is possible in this
apparatus in addition to the batch and semicontinuous modes
15   of operation can be implemented as desired in the cross-
flow filtration mode (Fig. 4) or in the form of dead-end
filtration (Fig. 3). Both process variants are described
in principle in the state of the art (Engineering Processes
for Bioseparations, Ed.: L.R. Weatherley, Heinemann, 1994,
20   135-165; Wandrey et al., Tetrahedron Asymmetry 1999, 10,
923-928).

A next development of the invention is concerned with a
process for the enzymatic transformation of organic
compounds by application of the reaction system according
25   to the invention. The process is preferably one involving
the preparation of an enantiomer-enriched organic compound,
preferably a chiral alcohol. The design of the process can
be worked out at the discretion of a person skilled in the
art on the basis of the reaction system that has been
30   described and the examples that are presented below. Under
the given boundary conditions, the conditions that are
otherwise known for the enzymatic conversion are set
appropriately.

A next aspect of the invention is concerned also with the
35   use of the reaction system according to the invention in a
process for the enzymatic transformation of organic

compounds or for the diagnosis or analysis of organic
compounds, preferably of alcohols. In further preferred
manner the reaction system according to the invention is,
as stated, employed in a process for the preparation of
5   enantiomer-enriched organic compounds, preferably of
alcohols.

Surprisingly, the formate dehydrogenase (FDH) derived from
Candida boidinii possesses very good stability in relation
to two-phase solvent systems. This will be investigated on
10   the basis of the experiments relating to the long-term
stability of the FDH derived from *C. boidinii* in various
solvent systems. In these experiments, according to
Comparative Example 1 and also Example 2 according to the
invention, a proportion of organic solvent of 10 % and 20 %,
15   respectively, with respect to the total volume was chosen.
In contrast with the water-soluble organic solvents (see
Comparative Example 1), which lead to a rapid deactivation
of the FDH derived from *Candida boidinii*, in the two-phase
system, particularly when use is made of the aforementioned
20   hydrocarbon components such as n-hexane, for example,
outstanding stability properties of the formate
dehydrogenase derived from *C. boidinii* (used in these
Examples in the form of the double mutant) were still
observed even after several days. Whereas, for example, in
25   the presence of acetone or DMSO the enzyme activity
declines within 24 hours by 35 % or 66 %, respectively, in
the presence of 20 vol.% hexane 90 % enzyme activity can
still be registered even after 3 days. The results with n-
hexane (Example 2) are reproduced in Fig. 1, represented
30   graphically, and in Table 3. The Comparative Examples with
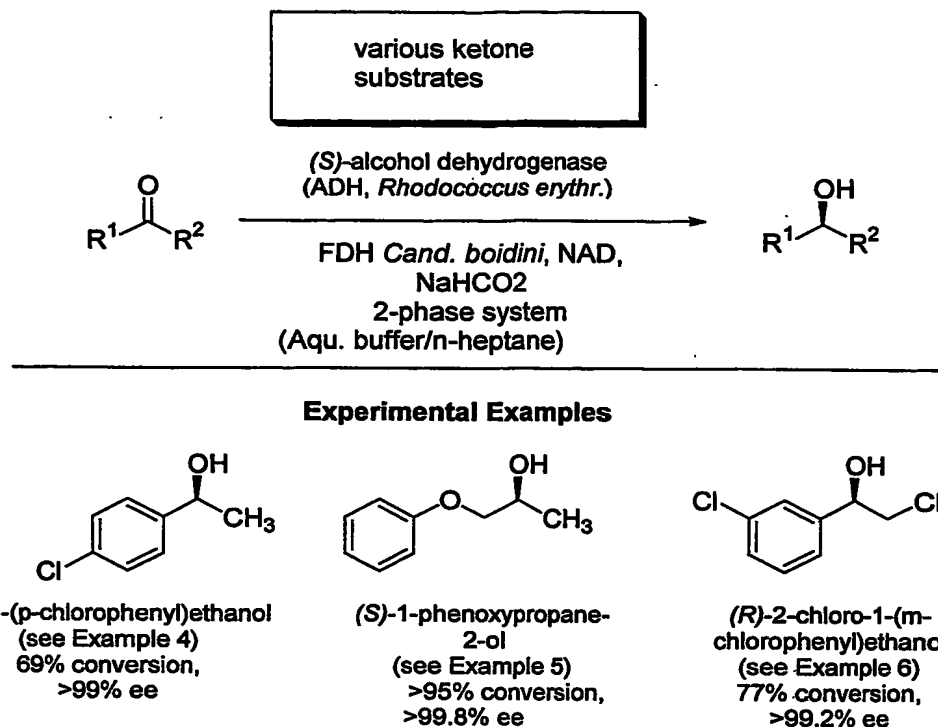other organic solvents are likewise recorded in Fig. 1 and
Table 1.

According to the invention, other organic solvents that are
not miscible with water and that consequently form two
35   phases can also be employed in the process that has been
described. For instance, it has been possible to achieve

very high stabilities also with n-heptane by way of organic
solvent component with a proportion of organic solvent of
20 %. The stability after 27 hours in this case was an
outstanding 99.8 % (see Example 3 and Table 4). In totally
5   surprising manner this activity is therefore still clearly
above the activity of a pure aqueous solution, pointing to
an unexpected stabilisation of the FDH derived from *Candida
boidinii* through the use of a 2-phase system (see also
Experimental Part, Example 3, Table 4 and Fig. 2).

10 Moreover, it is to be noted that the reaction system
according to the invention permits the enzymatic
conversions to be implemented successfully in the case
where use is made of 2-phase systems with a higher organic
proportion by volume. This is documented by the
15 experiments that were carried out using n-heptane with a
higher proportion of solvent (see Example 3, Table 4). In
the case of a proportion by volume of the organic solvent
n-heptane of 60 %, a high activity over a long period could
likewise be retained, as a residual activity of 82.8 %
20 after 27 hours makes clear. The results relating to the
long-term stability with varying proportions by volume of
organic solvent (according to Example 3, Table 4) are
represented graphically in the illustration of Fig. 2.

The present invention can be elucidated on the basis of the
25 example provided by the alcohol-dehydrogenase/NADH/
FDH/formic-acid system. The asymmetric synthesis of
alcohols was carried out by means of this reaction system,
starting from the corresponding ketone.

Scheme 2:

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                        ┌──────────────────────┐
                        │   various ketone     │
                        │   substrates         │
                        └──────────────────────┘
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              O   (S)-alcohol dehydrogenase   OH
              ‖   (ADH, Rhodococcus erythr.) |
        R¹───C───R²   ─────────────────────────────────→   R¹───C───R²
                               FDH Cand. boidini, NAD,
                                        NaHCO2
                                    2-phase system
                               (Aqu. buffer/n-heptane)

Experimental Examples

(S)-1-(p-chlorophenyl)ethanol (see Example 4) 69% conversion, >99% ee	(S)-1-phenoxypropane-2-ol (see Example 5) >95% conversion, >99.8% ee	(R)-2-chloro-1-(m-chlorophenyl)ethanol (see Example 6) 77% conversion, >99.2% ee

Processing of the reaction mixture was effected by

5   extraction with MtBE and concentration of the organic phase

by evaporation. The corresponding alcohol was obtained in

this way in a very simple manner in terms of apparatus in a

yield of 69 % and with an enantioselectivity of 99 %

(Example 4).

10 But outstanding enantioselectivities are also obtained with

the use of other ketones as starting materials. For

instance, the reduction of phenoxyacetone under these

reaction conditions results in an enantiopure product

quantitatively with > 99.8 % ee (Example 5).

15 But the reaction system according to the invention is also

suitable, moreover, for sterically demanding ketones. This

will be documented in exemplary manner on the basis of the

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example provided by α,m-dichloroacetophenone. This ketone
is substituted by a chlorine atom both on the methyl group
and on the aromatic ring. The biocatalytic reduction in
the 2-phase system here yields the desired product 2-
5   chloro-1-(m-chlorophenyl)ethanol, again with outstanding
enantioselectivity of > 99.2 % (Example 6). The conversion
here is around 77 %.

These high conversions and enantioselectivities are
surprising, not least for the reason that, by virtue of the
10 presence of organic solvents, often not only a diminution
of the enzyme activity (accompanied by a low conversion)
but also a change in enzyme properties with regard to
stereospecificity (accompanied by a diminution of
enantioselectivity) is to be observed.

15 In this context, however, the results of the experiments at
elevated substrate concentrations turned out to be
particularly surprising. These experiments were carried
out with p-chloroacetophenone as model substrate. If in
the above experiment at a substrate concentration of 10 mM
20 (this substrate concentration corresponds to the
concentration in the case of the experiments from the state
of the art) a conversion of 69 % is achieved (Example 4),
then this conversion - contrary to the widespread view that
at elevated substrate concentrations only diminished yields
25 can be achieved, by reason of inhibitions etc. - was able
to be increased with this type of reaction, starting now
from a concentration of 20 mM, and higher conversions of
75 % (at 40 mM) and 74 % (at 100 mM) could be achieved
(Examples 7, 8; Scheme 3; Fig. 5).

Scheme 3:
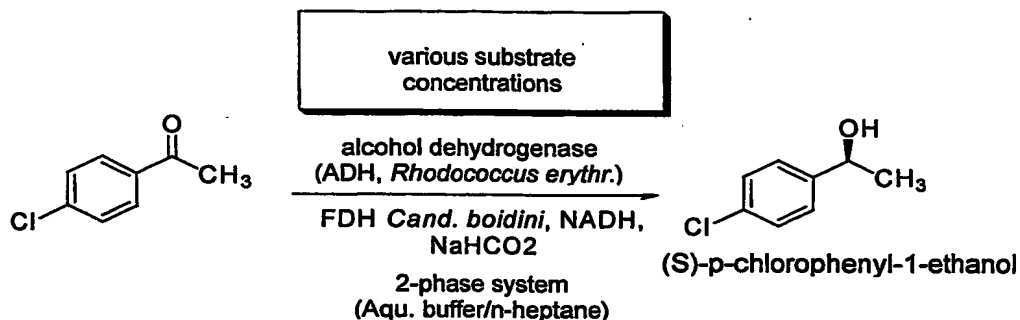
5   This process is consequently also suitable, in particular,
    for the enzymatic reduction of ketones at high substrate
    concentrations.

    A principal advantage of this process consists in its
    simplicity. For instance, no elaborate process steps are
10 included, and the process can be implemented both in batch
    reactors and continuously. Similarly, in contrast with
    earlier processes, no special membranes which separate the
    aqueous medium from the organic medium are required. The
    additions of surfactant which are required in some previous
15 processes also become unnecessary with this process. A
    further principal advantage consists in the first-time
    possibility of organising the enzymatic preparation of
    optically active alcohols in technically meaningful
    substrate concentrations of > 25 mM. These advantages
20 could not be deduced in obvious manner from the state of
    the art.

    The term 'enantiomer-enriched' designates the fact that one
    optical antipode is present in the mixture with its other
    one in a proportion amounting to > 50 %.

25 In the case where one stereocentre is present, the
    structures that are represented relate to both of the

possible enantiomers and, in the case where more than one
stereocentre is present in the molecule, to all possible
diastereomers and, with respect to one diastereomer, to the
possible two enantiomers of the compound in question which
5   are encompassed thereby.

The organism *C. boidinii* is deposited in the American Type
Culture Collection under number ATCC 32195 and is publicly
accessible.

The expression 'coupled enzymatic system' is understood to
10   mean, according to the invention, that an enzymatic
transformation of an organic compound takes place subject
to consumption of a cofactor and the cofactor is
regenerated *in situ* by a second enzymatic system (here the
FDH derived from *C. boidinii* or mutants thereof). As a
15   result, this leads to a diminution of the use of expensive
cofactors.

The documents of the state of the art that have been named
in this publication are considered as being jointly
encompassed by the disclosure.

Descriptions of the drawings:

Fig. 3 shows a membrane reactor with dead-end filtration.
The substrate 1 is transferred into the reactor chamber 3,
which exhibits a membrane 5, via a pump 2. Located in the
5 stirrer-driven reactor chamber, in addition to the solvent,
are the catalyst 4, the product 6 and unconverted substrate
1. Principally low-molecular product 6 is filtered off via
the membrane 5.

Fig. 4 shows a membrane reactor with cross-flow filtration.
10 The substrate 7 here is transferred via the pump 8 into the
stirred reactor chamber in which solvent, catalyst 9 and
product 14 are also located. Via the pump 16 a flow of
solvent is set which, via an optionally present heat-
exchanger 12, leads into the cross-flow filtration cell 15.
15 Here the low-molecular product 14 is separated via the
membrane 13. High-molecular catalyst 9 is subsequently
conducted back into the reactor 10 with the flow of
solvent, optionally again via a heat-exchanger 12,
optionally via the valve 11.

Experimental Part:

Example 1 (Comparative Examples of the FDH activities using
an FDH derived from *C. boidinii* (double mutant:
C23S/C262A))

5   2.72 g (0.8 mol/L) sodium formate and 1.14 g (0.1 mol/L)
dipotassium hydrogenphosphate trihydrate are weighed out
and dissolved in 40 mL fully demineralised H_2O. With
ammonia solution (25 %) and formic acid (100 %), or
corresponding diluted solutions, the pH value of the

10 solution is set to 8.2. Then the solution is transferred
into a 50 mL measuring flask and topped up with fully
demineralised H_2O. Separately from this, 71.7 mg
(4 mmol/L) NAD^+ trihydrate are weighed out and dissolved in
about 20 mL fully demineralised H_2O. With ammonia solution

15 (25 %) and formic acid (100 %), or corresponding diluted
solutions, the pH value of the solution is set to 8.2.
Then the solution is transferred into a 25 mL measuring
flask and topped up with fully demineralised H_2O.
Subsequently, in each case, 500 µL of the substrate

20 solution and also of the NADH solution are mixed in the
1 cm cell which is used for the measurement. After
addition of 10 µL of the enzyme solution, whereby a 10 %
solution of an organic solvent (see Table) in water finds
application by way of solvent, shaking is effected briefly,

25 the cell is placed into the photometer, and the recording
of data is started. The enzyme solution is firstly added
directly prior to the start of measurement. The activities
of the FDH derived from *C. boidinii* (double mutant:
C23S/C262A) are determined after certain periods of time by

30 the photometric detection of the reaction of NAD^+ to form
NADH. The photometric measurement was undertaken at a
temperature of 30 °C, at a wavelength of 340 nm and with a
measuring-time of 15 min. The results are represented
below in Table 1 and Table 2.

Tab. 1. Enzyme activity of the FDH derived from *C. boidinii* (double mutant: C23S/C262A) in U/mL as a function of solvent and time

Time	Butanol	MEK	DMSO	THF	Sulfolane	Aceto-nitrile
[d]	Activity [U/ml]	Activity [U/ml]	Activity [U/ml]	Activity [U/ml]	Activity [U/ml]	Activity [U/ml]
0.000	0.5262	0.0058	0.7965	0.8492	0.0028	0.7961
0.042	0.0006	0.0011	0.7880	0.4357	0.0003	0.4494
0.125			0.7794	0.0414		0.0840
1.097			0.2669			0.0008
2.035			0.2331			
2.896			0.2201			
5.927			0.1763			
7.885			0.1404			
9.948			0.1205			
13.073			0.0915			
14.892			0.0717			
16.875			0.0540			
19.938			0.0355			

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Tab. 2. Enzyme activity of the FDH derived from *C. boidinii* (double mutant: C23S/C262A) in U/mL as a function of solvent and time

Time	Acetone	Ethanol
[d]	Activity [U/ml]	Activity [U/ml]
0.000	0.8355	0.8491
0.042	0.7402	0.7689
0.750	0.5893	0.6367
1.000	0.5426	0.5933
1.875	0.3484	0.4687
2.760	0.2691	0.3510
3.781	0.2004	0.2814
4.646	0.1614	0.2240
5.875	0.1325	0.1736
6.778	0.0987	0.1486
7.792	0.0794	0.1277
8.729	0.0610	0.0998
11.750	0.0333	0.0536
13.726		0.0421

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Example 2 (Measurement of the FDH activities of the FDH
derived from *C. boidinii* (double mutant: C23S/C262A))

Determination of the activity was undertaken in accordance
with the instructions in Example 1, with hexane being used
5   as organic solvent component. The results are represented
below in Table 3.

Tab. 3.   Enzyme activity of the FDH derived from *C.*
boidinii (double mutant: C23S/C262A) in U/mL as a function
of hexane and time

Time	Hexane (10 %)	Hexane (20 %)
[d]	Activity [U/ml]	Activity [U/ml]
0.000	0.8364	1.0280
0.042	0.9572	0.9952
0.177	0.8223	1.1408
0.899	0.7892	0.9311
2.000	0.6242	0.9467
2.878	0.7654	0.9280

10

Example 3 (Measurement of the FDH activities of the FDH
derived from *C. boidinii* (double mutant: C23S/C262A))

Determination of the activity was undertaken in accordance
with the instructions in Example 1, with n-heptane being
15   used as organic solvent component. The results are
presented below in Table 4, the evaluation being given here
in percentage terms and the results at the individual
substrate concentrations relating in each case to the
initial activity which is denoted by 100 %.

Tab. 4. Enzyme activity of the FDH derived from *C. boidinii* (double mutant: C23S/C262A) in U/mL as a function of hexane and time

Time	n-Heptane (0 %)	n-Heptane (20 %)	n-Heptane (60 %)
[h]	Activity [%]	Activity [%]	Activity [%]
0	100.0	100.0	100.0
3	102.3	99.5	88.1
21	92.2	96.7	82.3
27	89.3	99.8	82.8

Example 4 (Conversion with p-chloroacetophenone)

To a solution consisting of p-chloroacetophenone (78.4 mg; 10 mM), sodium formate (50 mM) and NADH (2 mM) in 10 mL *n*-heptane and 40 mL of a phosphate buffer, 10.1 U of alcohol dehydrogenase (derived from *Rhodococcus erythropolis*) and 10 U of a formate dehydrogenase (FDH derived from *C. boidinii*, expression in *E. coli*, double mutant C23S/C262A) are added. The reaction mixture that has arisen is left to stir for 21 hours at 30 °C. Subsequently processing proceeds *via* extraction with 3 x 25 mL MTBE, and the collected organic phases are dried with sodium sulfate. The crude product resulting after removal of the solvent in a vacuum is examined with regard to conversion (by ^{1}H-NMR spectroscopic examination) and enantioselectivity (by chiral GC).

Conversion: 69 %

Enantioselectivity: > 99 % ee

Example 5 (Conversion with phenoxyacetone)

To a solution consisting of phenoxyacetone (76.0 mg;
10 mM), sodium formate (50 mM) and NADH (2 mM) in 10 mL *n*-
heptane and 40 mL of a phosphate buffer, 10.1 U of alcohol

5   dehydrogenase (derived from *Rhodococcus erythropolis*) and
10 U of a formate dehydrogenase (FDH derived from *C.
boidinii*, expression in *E. coli*, double mutant C23S/C262A)
are added. The reaction mixture that has arisen is left to
stir for 21 hours at 30 °C. Subsequently processing

10 proceeds *via* extraction with 3 x 25 mL MTBE, and the
collected organic phases are dried with sodium sulfate.
The crude product resulting after removal of the solvent in
a vacuum is examined with regard to conversion (by ^{1}H-NMR
spectroscopic examination) and enantioselectivity (by

15 chiral GC).

Conversion: > 95 %

Enantioselectivity: > 99.8 % ee

Example 6 (Conversion with 2,3′-dichloroacetophenone)

20 To a solution consisting of 2,3′-dichloroacetophenone
(102.7 mg; 10 mM), sodium formate (50 mM) and NADH (2 mM)
in 10 mL *n*-heptane and 40 mL of a phosphate buffer, 10.1 U
of alcohol dehydrogenase (derived from *Rhodococcus
erythropolis*) and 10 U of a formate dehydrogenase (FDH

25 derived from *C. boidinii*, expression in *E. coli*, double
mutant C23S/C262A) are added. The reaction mixture that
has arisen is left to stir for 21 hours at 30 °C.
Subsequently processing proceeds *via* extraction with 3 x
25 mL MTBE, and the collected organic phases are dried with

30 sodium sulfate. The crude product resulting after removal
of the solvent in a vacuum is examined with regard to
conversion (by ^{1}H-NMR spectroscopic examination) and
enantioselectivity (by chiral GC).

Conversion: 77 %

Enantioselectivity: > 99.2 % ee

Example 7 (Conversion with p-chloroacetophenone at 40 mM)

5   To a solution consisting of p-chloroacetophenone (78.4 mg; 10 mM), sodium formate (50 mM) and NADH (2 mM) in 2.5 mL *n*-heptane and 10 mL of a phosphate buffer, 10.1 U of alcohol dehydrogenase (derived from *Rhodococcus erythropolis*) and 10 U of a formate dehydrogenase (FDH derived from *C.*

10   *boidinii*, expression in *E. coli*, double mutant C23S/C262A) are added. The reaction mixture that has arisen is left to stir for 21 hours at 30 °C. Subsequently processing proceeds *via* extraction with 3 x 25 mL MTBE, and the collected organic phases are dried with sodium sulfate.

15   The crude product resulting after removal of the solvent in a vacuum is examined with regard to conversion (by ^{1}H-NMR spectroscopic examination) and enantioselectivity (by chiral GC).

Conversion: 75 %

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Example 8 (Conversion with p-chloroacetophenone at 100 mM)

To a solution consisting of p-chloroacetophenone (78.4 mg; 10 mM), sodium formate (50 mM) and NADH (2 mM) in 1 mL *n*-heptane and 4 mL of a phosphate buffer, 10.1 U of alcohol

25   dehydrogenase (derived from *Rhodococcus erythropolis*) and 10 U of a formate dehydrogenase (FDH derived from *C. boidinii*, expression in *E. coli*, double mutant C23S/C262A) are added. The reaction mixture that has arisen is left to stir for 21 hours at 30 °C. Subsequently processing

30   proceeds *via* extraction with 3 x 25 mL MTBE, and the collected organic phases are dried with sodium sulfate. The crude product resulting after removal of the solvent in

a vacuum is examined with regard to conversion (by ^{1}H-NMR
spectroscopic examination) and enantioselectivity (by
chiral GC).

Conversion: 74 %